Original Article
Effect of long-term dyslipidemia on arterial blood supply of inner ear

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Abstract: Objective: To verify whether the long-term dyslipidemia could cause the formation of atherosclerotic plaque in the inner ear and affect the inner ear blood supply, finally leading to the sudden sensorineural hearing loss (SSNHL). Methods: 100 patients with SSNHL were selected for this study as SSNHL group, while another 100 patients with normal hearing who were treated for polyp of vocal cord or deviation of nasal septum during the same period were selected as the control group. The indices of blood lipid (TC, TG, LDL-C and HDL-C) and blood viscosity were compared between two groups. Multi-variant Logistic regression analysis was performed to decide the risk factors for SSNHL. The rabbit atherosclerosis model was established using atherogenic diet and immune damage. Blood lipid indices include total cholesterol (TC) concentration, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG). Lipid stripe and atherosclerosis plaque of thoracic and abdomen aorta, AICA and vertebral artery were examined by HE staining. Sigmaplot 12 software was used for statistical analysis of all data. Results: The TC, TG and LDL-C of SSNHL group were significantly higher than those of control group, while the HDL-C was significantly lower than that of control group, the differences were statistically significant ($P<0.05$); the plasma viscosity, red cell assembling index, Erythrocyte deformation index and whole blood viscosity at low-shear of SSNHL group were also significantly higher than those of control group, and the differences were statistically significant ($P<0.05$); Multi-variant Logistic regression analysis showed LDL elevation is an important risk factor for SSNHL. The result of single factor logistic regression shows that TC ($P<0.01$), TG ($P<0.01$) and LDL-C ($P<0.01$) of test group were significantly higher than those of the control group. Moreover, the lipid stripe and atherosclerosis plaque were found in the thoracic aorta or abdomen of the test group (9/10), but no abnormal change was detected in AICA or vertebral artery of the test group. Conclusion: Dyslipidemia plays great important role in the pathogenesis of SSNHL, and LDL-C elevation is an important risk factor for SSNHL. The long-term dyslipidemia could hardly form the arterial embolization in the inner ear, which showed little correlation between long-term dyslipidemia and the inner ear blood supply, implying that the dyslipidemia might affect the SSNHL through other ways.

Keywords: SSNHL, dyslipidemia, high lipid model, inner ear blood supply, atherosclerotic plaque

Introduction

The sudden sensorineural hearing loss (SSNHL) is defined as sensorineural hearing loss of more than 30 dB, over three or more speech frequencies and it develops in less than 3 days. The diagnosis of this disease is based on anamnesis and audiometric testing. And there is no exact treatment method for successful recovery. So far, the risk factors leading to SSNHL development are not clear. Some studies reported that the occlusion, infection and stress of anterior inferior cerebellar artery (AICA) and vertebral artery were important risk factors for SSNHL [1, 2]. Among them, microcirculation disorder is the hotspot in SSNHL research; however, due to the anatomic structure of acoustic labyrinth, it's difficult to directly approve microcirculation disorder in micro-vessels of acoustic labyrinth for SSNHL patients.

Recently, many research evaluated the effect of different risk factors for cardiovascular disease and thrombus in the pathogenesis of SSNHL. It

is worth to note that the inner ear function is vulnerable to ischemia as the blood of inner ear is supplied by the terminal artery [3]. More importantly, dyslipidemia, which is one of the most important cardiovascular risk factors [4], shows a certain correlation with the incidence of SSNHL based on the increasing studies [5-7]. For example, there is a certain effect of lipid-lowering therapy in the treatment of SSNHL patients [7-10], which might be related to the disorder of the inner ear blood supply [11]. Moreover, another study showed that additional decreasing pathological lipoprotein and cholesterol concentrations in patients with SSNHL resulted in a statically significant hearing improvement, compared with the standard therapy [12].

However, the mechanism of dyslipidemia resulting in hearing loss hasn't been clearly stated until now. Previous studies reported that hyperlipoproteinaemia could cause SSNHL by influence on the blood vessel of the inner ear and the outer hair cells in the organ of corti. Hyperlipoproteinaemia may lead spasm of the spiralis modiolic artery and the vestibulocochlear artery. High cholesterol levels may cause changes in outer hair cell rigidity which affected the cell's ability to contract actively. In this study, we used the hyperlipidemic animal model to study whether the long-term dyslipidemia could cause the formation of atherosclerotic plaque in the supply artery of inner ear, thereby leading the development of SSNHL.

Methods

Subjects and grouping

From January, 2007 to December, 2012, 100 cases of SSNHL patients diagnosed and treated at department of otorhinolaryngology of Nantong People's Hospital were selected for this study as SSNHL group. There were 55 male patients and 45 female patients aged from 18-55 years old, with an average age of 45.12±13.86 years old. Inclusion criteria: patients had onset within 1 week; no previous medical treatment on SSNHL. Exclusion criteria: patients with Meniere disease, middle-ear disease, enlarged vestibular aqueduct syndrome (EVAS), acoustic neuroma, or accompanied with diabetes, hypertension, and patients with contradictions to prescribed drugs. Another 100 cases of patients with normal hearing who

were treated for polyp of vocal cord or deviation of nasal septum during the same period were selected as the control group. There were 58 male patients and 42 female patients aged from 16-58 years old, with an average age of (44.56±14.11) years old. The two groups of patients were comparable in general data, and the differences were not statistically significant (P>0.05).

Detection of blood lipid

In the morning on 2nd hospitalization day, 3 ml fasting venous blood were draw from all patients for further blood lipid detection. AU5800 Automatic Biochemic analyzer (Beckman Coulter, USA) and relevant regent kits was used to detect the blood lipid concentration with ELISA, the detection indices include triglyceride (TG), total cholesterol (TC), low-density lipoprotein (L-DL) and high-density lipoprotein (H-DL).

Detect of blood viscosity

In the morning on 2nd hospitalization day, 2 ml fasting venous blood were draw from all patients, and heparin was used for anticoagulation. Viscometer R80 (Beijing Shidi Scientific Instrument Corporation) was used for the detection of blood viscosity at 37°C according to the instruction of manufacture.

Animals and experiment design

All animal experiments were performed with the approval of the Animal Care Committee and complied with the Animal Management Rule of the Ministry of Public Health, People's Republic of China (Documentation 55, 2001). As is well known, the high lipid model of New Zealand rabbit is widely used to study the atherosclerosis caused by dyslipidemia, and can be formed through the high fat diet combined with arterial intimal injury or physical immunity [13-15]. Moreover, the New Zealand rabbit is also used in the study of the damage and ischemia of the inner ear [16, 17]. In this study, 20 adult male New Zealand rabbits weighing 2.34-2.88 Kg were randomly divided into experiment group (Test, n=10) and normal diet group (normal, n=10). At the beginning of this experiment, the blood lipid index of these rabbits were tested, including total cholesterol (TC) concentration, low density lipoprotein cholesterol (LDL-C), high
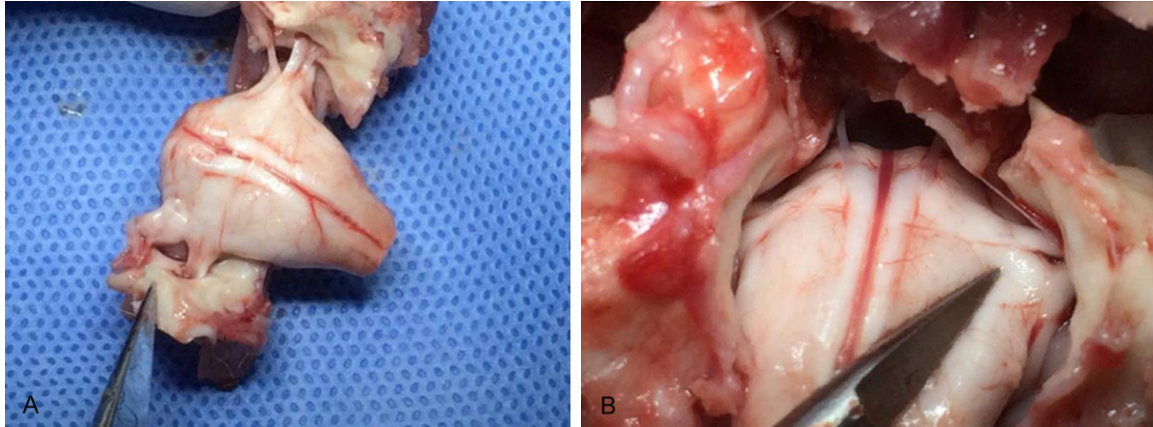
Figure 1. The AICA tissue sample of the rabbits. A: A brainstem of a rabbit from the experiment group. B: A tissue sample of AICA.

Table 1. Comparison of blood lipid between SSNHL group and control group

Group	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
SSNHL group	1.87±0.75*	4.98±0.97*	3.12±0.87*	1.18±0.37*
Control gourp	0.92±0.28	4.46±0.65	2.69±0.48	1.38±0.41

Note: *$P<0.05$, vs. control group.

density lipoprotein cholesterol (HDL-C) and tri-glycerides (TG). The experiment group was fed with an atherogenic diet (1% cholesterol, 5% lard, 10% yolk, and 84% standard animal diet) to induce hypercholesterolemia [13, 15, 18], while the normal group was fed with standard diet (100 g per rabbit per day). All the animals had free access to water.

Atherosclerosis model

After two weeks, all the rabbits from the experiment group were injected with solcoseryl albumin (Sangon, Shanghai, China, 250 mg/kg) from the marginal ear vein and led to immune damage, followed by 6 weeks of feeding constantly of previous diets. The rabbits from the control group were served as the experiment control and were injected with same amount of normal saline. At the end of the experiment, the adult male rabbits were sacrificed by intravenous injection of air.

AICA tissue preparation

The sacrificed rabbits received antero-lateral median incision from the trachea to avoid the common carotid artery. The tissue was separated by orceps, and the tracheas as well as esophagus were pulled to one side. The muscular tissue attached to skull base was also separated by blunt dissection to expose the skull base. Then, a bone window (4 mm × 4 mm, with diamond drill) was opened at the skull base, and the dura mater was carefully pierced by a probe, followed by the outflow of cerebrospinal fluid to expose AICA, which was taken out with the diamond drill (**Figure 1**).

HE staining

Thoracic and abdomen aorta, AICA and vertebral artery were harvested from each rabbit and immediately fixed in 10% buffered formalin overnight. The tissue samples were embedded in paraffin wax and cut into 3-5 μm sections. The sections were flattened, mounted and heated on blank glass slides. The sections were stained with hematoxylin and eosin (HE) after de-paraffinization and dehydration, according to the procedures.

Statistical analysis

All statistical analyses were processed by Sigmaplot 12. The data were expressed as mean ± SD, and the comparison between groups were examined by T test. Count data were expressed as percentage, and the comparison between groups was examined by Chi-square test Rank sum test was used if the data were not normally distributed. Multi-vari-

Table 2. Comparison of blood viscosity between SSNHL group and control group

Group	Plasma viscosity (mPa.S)	Red cell assembling index	Erythrocyte deformation index (Tk)	Whole blood viscosity at low-shear (mPa.S)
SSNHL group	1.58±0.37*	5.41±1.21*	0.79±0.03*	50.98±2.09*
Control group	1.45±0.37	2.76±0.15	0.74±0.09	38.27±2.68

Note: *P<0.05, vs. control group.

Table 3. Univariant Logistic analysis on the indices of blood lipid

Variants	β	SE	Wald	P	OR	OR 95% CI
TG	0.097	0.198	0.243	0.618	1.100	0.742~1.632
TC	0.407	0.162	6.175	0.012	1.498	1.084~2.063
HDL-C	0.587	0.209	7.649	0.005	1.799	1.186~2.758
LDL-C	-1.010	0.416	5.851	0.015	0.359	0.158~0.819

Table 4. Result from multi-variant Logistic regression analysis

Variant	β	SE	Wald	P	OR	OR 95% CI
LDL-C	0.596	0.219	7.607	0.005	1.851	1.188~2.844

Table 5. Body weight of the rabbits in the two groups (kg)

Groups	Number of rabbits	0 wk (baseline)	2 wk	4 wk	6 wk	8 wk
Normal	5	2.64±0.18	2.69±0.16	2.74±0.17	2.83±0.16	2.88±0.15
Test	10	2.62±0.17	2.72±0.19	2.98±0.14	3.25±0.22	3.46±0.19

ant analysis was analyzed by Logistic multi-variant regression analysis, P<0.05 was considered to be statistically significant.

Results

The comparison of blood lipid between two groups of patients

From **Table 1**, we can see the concentrations of TC, TG and LDL-C of SSNHL group were significantly higher than those of control group, the differences were statistically significant (P<0.05).

Comparison of blood viscosity between two groups of patients

From **Table 2**, we can see the plasma viscosity, red cell assembling index, Erythrocyte deformation index and whole blood viscosity at low-shear of SSNHL group were significantly higher than those of control group, the differences were statistically significant (P<0.05).

Univariant analysis

Univariant Logistic analysis was performed with TC, TG, LDL and HDL as independent variants while SSNHL as dependent variant, and the result showed that TC, LDL and HDL were the risk factors for SSNHL, see **Table 3**.

Multi-variant Logistic analysis

The risk factors concluded from Univariant Logistic analysis were analyzed by Multi-variant Logistic regression analysis. At a level of α=0.2, TC, LDL and HDL as independent variables and SSNHL as dependent variable, Logistic regression was performed by the maximum likelihood estimation method, and the result suggested that LDL-C was the relevant risk factor for SSNHL, see **Table 4**.

Detection of lipid plaques in inner ear artery of rabbits

As shown in **Table 5**, the body weight of the experiment (test) group showed an obvious increase compared to the control (normal) group. In addition, the significant differences between the experiment and control group in TC (at the 6th week), TG (at the 4th week), and LDL-C (at the 2nd week) were observed, while the HDL-C showed no significant difference throughout the experiment (**Table 6**). Moreover, the lipid stripe and atherosclerosis plaque were found in the thoracic aorta or abdomen of the experimental animals (9/10), while no such formation was observed in the control group (**Table 7**). It is worthy to note that even the lipid stripe and atherosclerosis plaque were found in the thoracic aorta or abdomen of the experimental animals, but no abnormal change was detected in AICA or vertebral artery (**Figure 2**).

Table 6. Lipid profile of serum in the two groups (mmol/L)

	Groups	n	0 wk	2 wk	4 wk	6 wk	8 wk
TC	Normal	5	1.28±0.23	1.30±0.09	1.39±0.20	1.34±0.29	1.31±0.23
	Test	10	1.36±0.22	1.44±0.20	1.59±0.24	1.79±0.31**	1.98±0.41*
TG	Normal	5	0.79±0.11	0.82±0.35	0.88±0.31	1.20±0.13	1.00±0.27
	Test	10	0.80±0.18	0.81±0.21	1.38±0.35**	1.53±0.34	1.79±0.31*
LDL-C	Normal	5	0.87±0.21	0.81±0.21	0.75±0.24	0.58±0.21	0.62±0.10
	Test	10	0.85±0.14	1.28±0.43**	2.78±1.05*	3.77±1.26*	4.12±1.51*
HDL-C	Normal	5	0.79±0.21	0.84±0.16	0.89±0.29	0.88±0.25	0.87±0.31
	Test	10	1.01±0.27	0.97±0.28	1.04±0.20	0.97±0.35	1.06±0.31

TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol. Data are expressed as mean ± SD, *$P<0.05$; **$P<0.01$ compared with normal.

Table 7. Lipid plaque and fatty streak in the two groups

	Thoracic aorta	Abdominal aorta	Vertebral artery	AICA
Test (n=10)	9	9	0	0
Normal (n=5)	0	0	0	0

Discussion

Although the etiology of SSNHL hasn't been clearly stated until now, different theories about its etiology and risk factors of SSNHL are a topic of debate across the literature [19]. For example, some clinical research indicated that risk factors of SSNHL overlap those of cardiovascular disease, such as hyperfibrinogenemia and smoking [12, 20]. On the other side, a clinical study of 86 subjects in China showed that the levels of total cholesterol (TC), triglyceride (TG), and lipoprotein A (Lp(a)) were significantly higher in patients with SSNHL than in control subjects. Moreover, Oreskovic reported that patients with SSNHL had significantly higher plasma concentrations of cholesterol and low-density lipoprotein cholesterol, compared with controls [6]. Recently, in order to add to the clinical evidence of this problem and to avoid selection bias, we carried out a retrospective study of the SSNHL patients between 2007 and 2012 in a medium-sized clinical hospital of Nantong, China. The result showed that the TC, TG and LDL-C in SSNHL group were significantly higher than those of control group, while the HDL-C was significantly lower than that of control group, the differences were statistically significant ($P<0.05$). In addition, Multivariant Logistic regression analysis indicated that the elevation of LDL was the main risk factor for the pathogenesis of SSNHL. Based on

these, we can see dyslipidemia plays a significant role in the pathogenesis of SSNHL. We speculated the elevation of blood lipid induced high viscosity of blood, which is complied with the result of the present study that the blood viscosity of SSNHL group was significantly higher than that of the control group ($P<0.05$), and further result in the dysfunction of inner ear microcirculation. Besides, blood lipid can attach to the surface of red cells and blood platelet, which may decrease the charge-carrying ability of red cells and enhance the adhesion between red cells, moreover, the increase of TC can result in the sclerosis of erythrocytes and the impact the oxygen-carrying ability and deformability of erythrocytes. The metabolic residuals of TG can directly destroy blood vessels and promote the formation of thrombus.

As we know that hyperlipoidemia could promote the occurrence of coronary artery disease through the way of atherosclerosis. Labyrinth arteries, which supply the blood of the inner ear, show a great variation in the origin of articular branches, and most of them are the branches of AICA except occasionally direct from the basilar artery [21, 22]. Since the previous hyperlipoidemia studies focused mainly on coronary artery, the correlation between the long-term dyslipidemia and ACIA is still not clear. An animal experiment showed endothelial dysfunction in cerebral arterioles were significantly increased in apoE (-/-) mice on the high-fat diet >6 months compared with the control group [23]. Another animal experiment found that blood flow in the cochleas was reduced significantly in hypertensive rats exposed to an atherogenic diet for six months compared to that of normotensive or hypertensive control animals. It is generally accept-
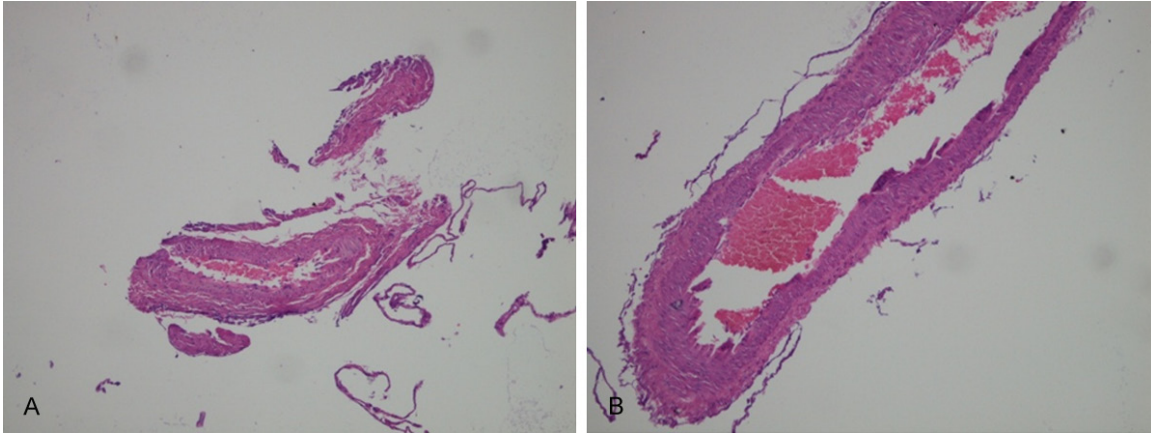
Figure 2. The light microscopic photographof artery tissues. A: AICA; B: Vertebral artery.

ed that Nitric oxide, a potent vasodilator plays an extremely important role in the blood supply of inner ear [25], while LDL cholesterol can impair NO release, influencing the blood supply of inner ear [26]. Obviously, these studies prompted that the dyslipidemia could reduce the blood supply of small arteries and microcirculation. However, there is no research on the problem of whether the long-term dyslipidemia could cause the lipid plaque formation in the blood supply arteries of inner ear, such as AICA etc. Therefore, we used the New Zealand rabbit model of hyperlipidemia combined with immune endothelial-cell injury to study their correlations. The results showed that the body weight and blood lipid of the experiment group with high fat diet were significantly higher than those of the control group, and the expression of atherosclerosis in the thoracic aorta or abdomen was also observed in the experimental animals (9/10). It is worthy to note that even in the case of large atherosclerosis caused by dyslipidemia, no abnormal change in the inner ear blood supply was detected under the light microscopy, which prompt that the dyslipidemia affected the SSNHL through other ways.

Unlike other animal species, the regeneration ability of vestibular and cochlear hair cells of mammals is completely limited after birth [2], and many reasons such as diseases, noise and drugs can result in the degeneration and necrosis of the inner hair cells, leading to the SSNHL in mammals at last. Research has proved that the envelope protein is contributed to the function of hair cells in the inner ear as an important material base, in which the prestin is one of most important envelope proteins, and is

mainly adjusted by cholesterol [27]. In addition, the activity of hair cells was reduced when they were cultured *in vitro* with high cholesterol medium, which could be caused by the significant amount of cholesterol aggregated in the hair cell membrane [28]. In the contrary, the activity of hair cells increased after the cholesterol was washed from the cell membrane [29]. And next step, we will use the dyslipidemia of animal model to study the effect of long-term dyslipidemia on the hair cell dysfunction of the inner ear in the next step.

Conclusion

The retrospective study of SSNHL patients showed that LDL is the main risk factor for the pathogenesis of SSNHL. However, the animal experiment suggested that the long-term dyslipidemia was hard to form the arterial embolization in the blood supply related arteries of inner ear, which showed little correlation between the long-term dyslipidemia and the inner ear blood supply, and implied us that the dyslipidemia might affect the SSNHL through other ways.

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Disclosure of conflict of interest

None.

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